VOLUME 31 · NUMBER 27 · SEPTEMBER 20 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Intrachromosomal Amplification of Chromosome 21 Is Associated With Inferior Outcomes in Children With Acute Lymphoblastic Leukemia Treated in Contemporary Standard-Risk Children's Oncology Group Studies: A Report From the Children's Oncology Group

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Published online ahead of print at www.jco.org on August 12, 2013.

Supported by Children's Oncology Group (COG) Chair's Grant No. U10 CA98543, COG Statistical Center Grant No. U10 CA98413, and COG Specimen Banking Grant No. U24 CA114766 from the National Cancer Institute.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/13/3127w-3397w/$20.00

DOI: 10.1200/JCO.2013.49.1308

ABSTRACT

Purpose
Five-year overall survival (OS) for children with B-cell precursor acute lymphoblastic leukemia (B-ALL) exceeds 90% with risk-adapted therapy. Age, initial WBC count, genetic aberrations, and minimal residual disease (MRD) are used for risk stratification. Intrachromosomal amplification of a region of chromosome 21 (iAMP21; three or more extra copies of *RUNX1* on an abnormal chromosome 21) is a recently identified recurrent genomic lesion associated with inferior outcome in some studies. We investigated the impact of iAMP21 in a large cohort treated in contemporary Children's Oncology Group (COG) ALL trials.

Patients and Methods
Fluorescent in situ hybridization for specific genetic aberrations was required at diagnosis. MRD was measured by flow cytometry at end induction. Outcome was measured as event-free survival (EFS) and OS.

Results
iAMP21 was found in 158 (2%) of 7,793 patients with B-ALL age ≥ 1 year; 74 (1.5%) of 5,057 standard-risk (SR) patients, and 84 (3.1%) of 2,736 high-risk (HR) patients. iAMP21 was associated with age ≥ 10 years, WBC less than 50,000/μL, female sex, and detectable MRD at day 29. Four-year EFS and OS were significantly worse for patients with iAMP21 and SR B-ALL, but iAMP21 was not a statistically significant prognostic factor in HR patients. There was no interaction between MRD and iAMP21. Among SR patients, day 29 MRD ≥ 0.01% and iAMP21 were associated with the poorest EFS and OS; absence of both was associated with the best outcome.

Conclusion
iAMP21 is associated with inferior outcome in pediatric B-ALL, particularly SR patients who require more intensive therapy and are now treated on HR COG ALL protocols.

J Clin Oncol 31:3397-3402. © 2013 by American Society of Clinical Oncology

INTRODUCTION

The outcomes for childhood acute lymphoblastic leukemia (ALL) have steadily improved such that 5-year overall survival (OS) rates now exceed 90%.[1] One of the factors contributing to this success has been the application of risk-adapted therapy, whereby the intensity of therapy is tailored to the predicted risk of relapse. In addition to clinical features and measurements of early treatment response, the presence or absence of specific sentinel genetic lesions present in leukemic blasts plays an essential role in determining prognosis and stratifying ALL therapy. Hyperdiploidy with specific extra chromosomes or an *ETV6-RUNX1* rearrangement predict an excellent outcome,[2] but hypodiploidy with less than 44 chromosomes,[3] the presence of the Philadelphia chromosome (Ph^+) with *BCR-ABL1* fusion,[4,5] or an *MLL* rearrangement are associated with an inferior outcome.[6,7]

Children with ALL often undergo blast fluorescent in situ hybridization (FISH) testing at diagnosis

for the presence of the *ETV6-RUNX1* fusion, which led to identification of intrachromosomal amplification of a region of chromosome 21 (iAMP21) that included multiple copies of *RUNX1* as a recurrent genetic lesion. First described in 2003,[8] iAMP21 occurs in 1% to 3% of children with ALL and has been associated with a poor outcome.[9-11] However, most reports of poor outcome published to date are based on small patient numbers and/or on treatment regimens used before 2000 that were less effective than current therapies.

Amplification of *RUNX1* occurs on a rearranged chromosome 21. The rearrangement is heterogeneous and complex and varies considerably among patients, with amplification occurring on both the short (p) and/or long (q) arms of the chromosome.[12] *RUNX1* is located in the common region of amplification; however, it is not known whether extra copies of *RUNX1* contribute to the poor prognosis associated with iAMP21. Expression arrays have shown that *RUNX1* is neither overexpressed nor mutated in children with this aberration.[13] Children with the iAMP21 aberration tend to be older, with a median age of 9 years, have a common or pre-B immunophenotype, and have low platelet counts and low presenting WBC counts.[10,11]

The presence of detectable levels of minimal residual disease (MRD) at early time points in therapy for childhood ALL is the most robust predictor of outcome and a key variable used for risk classification.[14,15] Both the presence and absence of specific sentinel cytogenetic lesions and MRD are used to risk stratify children with ALL treated in Children's Oncology Group (COG) clinical trials.[2,14] We report the outcome of children with iAMP21 treated in contemporary COG ALL trials and the influence of National Cancer Institute (NCI) risk group and MRD on the prognostic significance of this aberration.

PATIENTS AND METHODS

Between December 29, 2003, and September 2, 2011, 5,057 children, adolescents, and young adults with newly diagnosed NCI standard risk (SR; age $>$ 1 year and $<$ 10 years and initial WBC $<$ 50,000/μL) and 2,736 with newly diagnosed NCI high-risk (HR; age 10 to 30 years or initial WBC $\geq$ 50,000/μL and any age) B-cell ALL (B-ALL) were enrolled onto the COG AALL0331 and AALL0232 clinical trials, respectively. All patients were also enrolled onto the companion ALL risk classification and biology study, AALL03B1, which included a standard battery of cytogenetic and FISH studies and early disease response assessments that were used in conjunction with clinical features to assign risk and allocate postinduction therapy.[2] All studies were approved by the NCI and by the institutional review board of each participating institution. Informed consent was obtained from the patients and/or families. Treatment on AALL0331 and AALL0232 consisted of a three- (AALL0331) or four-drug (AALL0232) induction, with postinduction therapy based on early response and established prognostic cytogenetic features that included randomized treatment interventions (Data Supplement). The postinduction therapy backbone used for SR patients was less intensive than that used for HR patients. A low-risk subset of SR patients was also defined postinduction on the basis of favorable cytogenetics and early response, and this subgroup of patients also received less intensive therapy. Therapy was not altered for patients with iAMP21, and patients with this aberration were not recommended to undergo hematopoietic stem-cell transplantation (HSCT) in first remission.

FISH analyses of bone marrow samples that used probes for the centromeres of chromosomes 4, 10, and 17 were required throughout the course of the AALL03B1 study. This was done initially in COG central or approved local laboratories (2003 to 2006) and later (2007 to 2011) exclusively in COG-approved local cytogenetic laboratories. Testing for *ETV6-RUNX1*, *BCR-ABL1*, and *MLL* rearrangements was done by reverse transcriptase polymerase chain reaction (RT-PCR) or FISH in one of two central reference laboratories (2003 to 2006) or by FISH in COG-approved local laboratories (2007 to 2011). Standard karyotype analysis was performed in COG-approved local laboratories. All approved local laboratory results were centrally reviewed. Ascertainment of iAMP21 may have been incomplete before 2007 because *ETV6-RUNX1* was primarily assessed centrally by RT-PCR. Classification as iAMP21 required three or more extra copies of *RUNX1*on a single abnormal chromosome 21 ($\geq$ five total *RUNX1* signals). If metaphase FISH was not possible, iAMP21 was identified as multiple copies of *RUNX1* clustered (cosegregating) in at least some of the interphase nuclei. MRD was measured in bone marrow in one of two central COG reference laboratories by flow cytometry in all patients at the end of induction (day 29).[14]

Table 1. Clinical Characteristics of Patients According to iAMP21 Status

Characteristic	iAMP21 (%)	Non-iAMP21 (%)	*P*
Age $\geq$ 10 years	50	23	$<$ .001
WBC $<$ 50,000/μL	95	85	$<$ .001
NCI high risk	53	34	$<$ .001
Female	55	46	.0238

NOTE. Intrachromosomal amplification of a region of chromosome 21 (iAMP21) was found in 158 (2%) of 7,793 patients; 74 (1.5%) of 5,057 patients were standard risk; 84 (3.1%) of 2,736 patients were high risk.
Abbreviation: NCI, National Cancer Institute.

Statistical Analyses

Patients with both *MLL* rearrangement and a slow early response to treatment (day 15 marrow $\geq$ 5% blasts and/or day 29 MRD $\geq$ 0.1%), hypodiploidy (DNA index $<$ 0.81 or chromosome number $<$ 44), or Ph$^+$ ALL were not eligible for postinduction therapy on either AALL0232 or AALL0331 and were excluded from all analyses in this report. Event-free survival (EFS) was calculated as the time from diagnosis to first event (induction failure, relapse at any site, secondary malignancy, or death as a result of any cause) or last contact for patients who did not have an event. OS was defined as the time from diagnosis to death or last contact in patients who were alive. Data current as of December 31, 2011, were used in this report. The log-rank test was used to compare survival curves (one-sided test). EFS and OS estimates were calculated by the method of Kaplan and Meier,[16] and SEs of estimates were obtained by using the method of Peto.[17] χ^2 and Fisher's exact tests were used for comparison of proportions. Alpha was set at 5% for all comparisons. All data analyses were performed by using the SAS System (SAS Online Doc 9.2, 2007; SAS Institute, Cary, NC) and R (R Foundation for Statistical Computing, 2010, Vienna, Austria; http://www.R-project.org/).

RESULTS

iAMP21 was identified in 158 (2%) of 7,793 patients with B-ALL including 74 (1.5%) of 5,057 SR patients and 84 (3.1%) of 2,736 HR patients. No patients with iAMP21 had trisomies of chromosomes 4 and 10, *ETV6-RUNX1*, *MLL* rearrangements, or *BCR-ABL1*. Several diagnostic features differed between patients with and without iAMP21. Those with iAMP21 were significantly more likely to be NCI HR, to be $\geq$ 10 years old, to have WBC $<$ 50,000/μL, and to be female (Table 1).

Both EFS and OS were inferior in patients with iAMP21 compared with patients without iAMP21. In analyses including both SR and HR patients, 4-year EFS for those with and without iAMP21 was 72.7% $\pm$ 5.8% versus 88.1% $\pm$ 0.6%, respectively ($P <$ .001), and 4-year OS was 87.6% $\pm$ 4.4% versus 94.0% $\pm$ 0.4%, respectively (P = .0184; Fig 1).

Comparisons of SR patients with and without iAMP21 also showed significantly worse EFS (4-year EFS, 72.7% $\pm$ 7.5% *v* 92.0% $\pm$

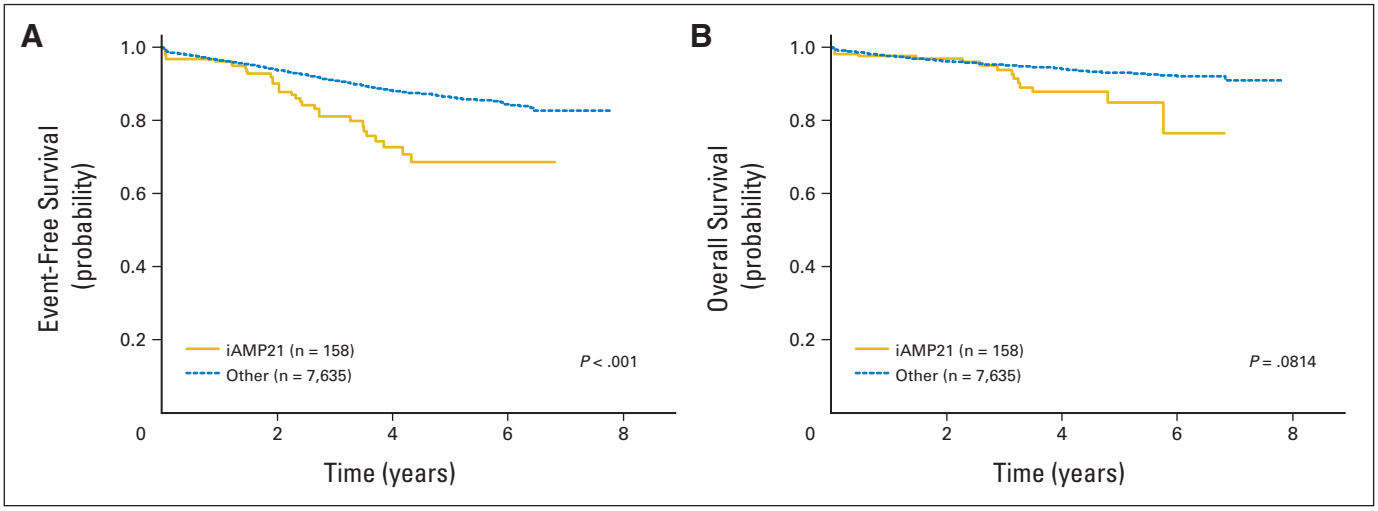

Fig 1. Outcomes for all pooled standard-risk and high-risk patients according to intrachromosomal amplification of a region of chromosome 21 (iAMP21) status. (A) Event-free survival for all patients and (B) overall survival for all patients according to iAMP21 status.

0.6%; $P < .001$) and OS (4-year OS, 89.9% ± 5.2% *v* 96.9% ± 0.4%; $P < .001$) for patients with iAMP21 than for those without iAMP21 (Figs 2A and 2B). However, there were no significant differences in EFS (4-year EFS, 72.5% ± 9.2% *v* 80.8% ± 1.1%; $P = .6670$) and OS (4-year OS, 85.9% ± 7.2% *v* 88.6% ± 0.9%; $P = .9901$) between HR patients with and without iAMP21 (Figs 2C and 2D).

Patients with iAMP21 were more likely to be MRD positive at end induction than were those without iAMP21 using cutoffs of ≥ 0.01% (42% *v* 21%; $P < .001$) or ≥ 1.0% (8% *v* 4%; $P = .0078$). This was also true for both SR (43% *v* 18%; $P < .001$) and HR patients (40% *v* 27%; $P = .0344$) using a cutoff of ≥ 0.01%. Outcome comparisons were also made examining iAMP21 status and end induction MRD (< 0.01% *v* ≥ 0.01%). No interaction between MRD and iAMP21 was found in any of the comparisons. Among all (pooled SR and HR) patients with MRD ≥ 0.01%, iAMP21 patients had a worse EFS compared with those without iAMP21 (Table 2 and Data Supplement). There was also a significant difference in OS based on MRD positivity at the end of induction and iAMP21 status ($P < .001$; Table 2 and Data Supplement), with MRD-negative patients without iAMP21 having the best OS (96.6% ± 0.4%) and MRD-positive patients with iAMP21 having the worst OS (82% ± 7.8%). However, when adjusted for MRD positivity, there was no difference in OS on the basis of iAMP21 status.

In an analysis restricted to SR patients only, iAMP21 status was significantly predictive of EFS and OS in MRD-positive and MRD-negative subsets (Table 2 and Data Supplement). Those who were MRD positive with iAMP21 had significantly worse EFS than MRD-positive patients without iAMP21 (4-year EFS, 59.6% ± 12.0% *v* 85.2% ± 1.9%, respectively; $P < .001$). SR patients who were MRD negative and iAMP21 positive had worse EFS than SR MRD-negative patients without iAMP21 (4-year EFS, 82.2% ± 9.0% *v* 94.2% ± 0.5%, respectively; $P < .001$). OS also differed significantly on the basis of MRD and iAMP21 status ($P < .001$; Data Supplement). Although the OS for MRD-positive SR iAMP21 patients was not statistically significantly worse than that of MRD-positive patients without iAMP21 (4-year OS, 85.2% ± 9.1% *v* 93.8% ± 1.3%, respectively; $P = .082$), OS was significantly inferior for MRD-negative SR patients with iAMP21 *v* those without (4-year OS, 93.0% ± 6.2% *v* 98.1% ± 0.3%, respectively; $P = .0094$). However, in contrast to SR patients, iAMP21 was not significantly predictive of EFS or OS among HR MRD-negative or MRD-positive subsets (Table 2 and Data Supplement).

Multivariate Cox regression analyses were run, including iAMP21 status, age at diagnosis (≥ 10 years), WBC at diagnosis (≥ 50,000/μL), day 29 MRD positivity (> 0.01%), and presence of favorable genetics (*ETV6-RUNX1* or trisomies of chromosomes 4 and 10). In the pooled analysis (SR and HR patients), iAMP21 did not make a significant contribution (hazard ratio, 1.365; $P = .105$) in the presence of the other variables, all of which were statistically significant (Table 3). This was also true when the HR patients were analyzed separately (iAMP21 hazard ratio, 0.844; $P = .5835$). However, for SR patients, in addition to day 29 MRD and presence of favorable cytogenetics, iAMP21 was also statistically significant in multivariate analyses (hazard ratio, 2.277; $P = .0012$).

Thirty-two of the 158 iAMP21 patients had events: SR (18 events) and HR (14 events; Table 4). These events included two induction failures (M3 marrow on day 29 induction), three induction deaths, 25 relapses, one secondary malignancy, and one remission death. Relapses comprised 88.8% of all events in SR patients and 64.4% of all events in HR patients. Given the small numbers, there were no obvious differences in the patterns of events between SR and HR patients with iAMP21. The distribution of sites of relapse among patients with and without iAMP21 was nearly identical, with isolated marrow relapses being most frequent in both groups (Data Supplement).

DISCUSSION

This study of 158 patients with iAMP21 (of nearly 8,000 total patients) is the largest reported to date. As in previous reports,[8,10] iAMP21 was found in 2% of COG patients with B-ALL older than age 1 year. By using contemporary COG therapy, patients with ALL with iAMP21 have a significantly worse EFS and OS than patients without iAMP21. This is largely explained by poor outcomes for SR patients with ALL with iAMP21, the majority of whom were treated with less intensive

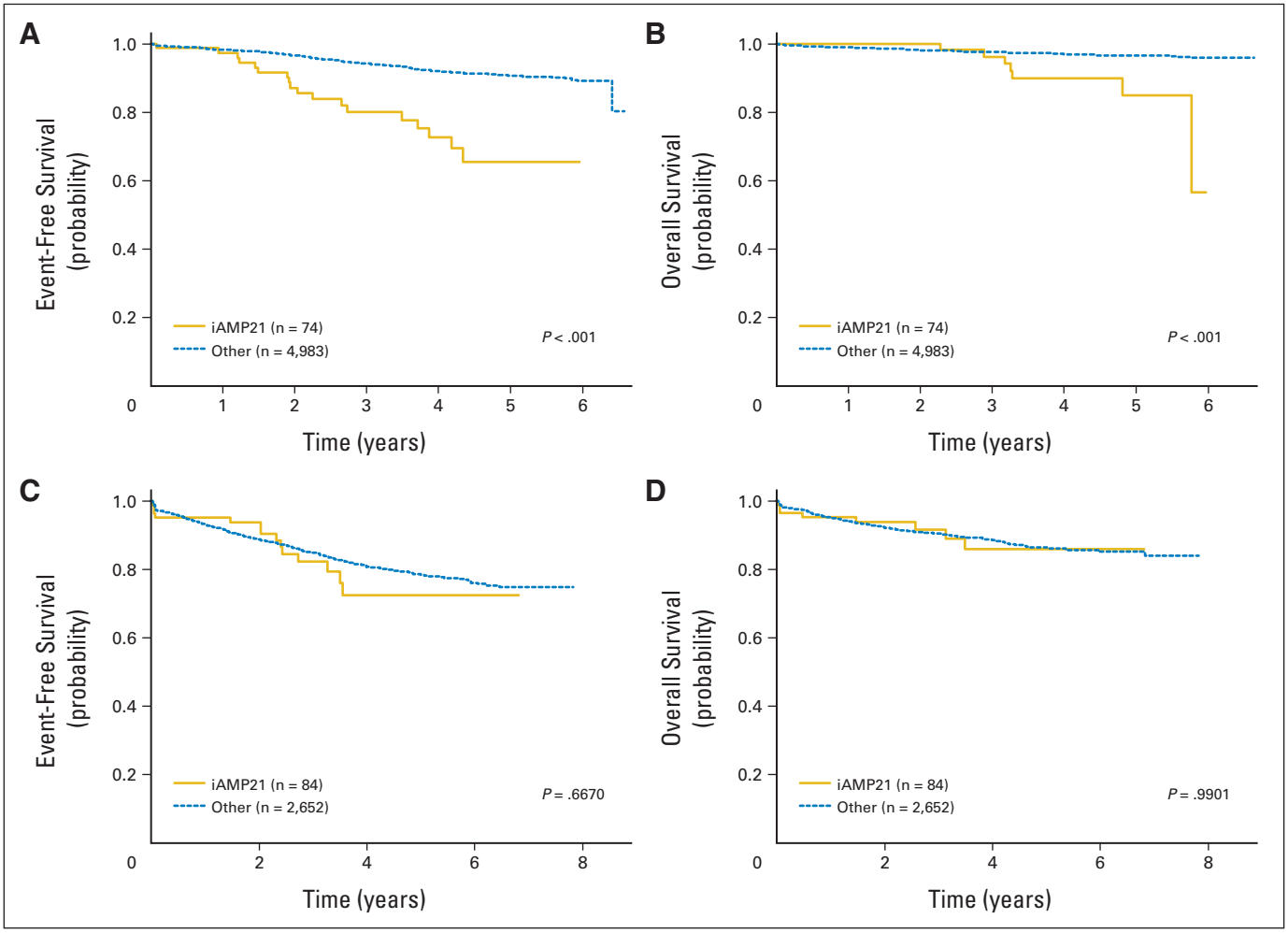

Fig 2. Outcomes for standard-risk and high-risk patients according to intrachromosomal amplification of a region of chromosome 21 (iAMP21) status. (A) Event-free survival and (B) overall survival in standard-risk patients; (C) event-free survival and (D) overall survival in high-risk patients.

therapy. In contrast, iAMP21 did not result in a significantly inferior EFS or OS for NCI HR patients, including the MRD $\geq$ 0.01% or MRD $<$ 0.01% subsets. Similar to previous reports,[9-11] iAMP21 patients in these studies were older (50% age $\geq$ 10 years) and had relatively low WBC (95% $<$ 50,000/μL). Unlike patients in some previous reports,[9,18] our patients tended to be female (55%). None of these clinical features help to identify iAMP21 patients. FISH for *RUNX1* remains the most reliable routinely performed method to identify iAMP21, although it can also be readily detected by single nucleotide polymorphism arrays or other arrays used to determine DNA copy number alterations that may enter routine clinical practice in coming years. Metaphase cytogenetics may suggest the presence of iAMP21 when there is an abnormal chromosome 21 or monosomy 21 and a marker chromosome, but these aberrations also can occur in patients who do not have iAMP21.

The United Kingdom ALL97/99 studies reported a 5-year EFS of 26% and 5-year OS of 69% for 28 patients with iAMP21,[18] and the Austrian and German ALL Berlin-Frankfurt-Munster (ALL-BFM) 86, 90, 95, and 2000 trials reported a 6-year EFS of 38% and OS of 66% for 29 iAMP21 patients.[9] Both of these studies included relatively small numbers of patients with iAMP21, most of whom were treated with less intensive therapy than that included in standard regimens used by those groups today. In our study, which included more than five times as many patients with iAMP21 as these reports, outcomes were much better with 4-year EFS of 73% for pooled SR and HR iAMP21 patients and OS of 88%. Although the outcomes in the COG trials are better than those previously reported for this group, additional follow-up is needed to determine whether outcome results will decline with longer follow-up. However, in these contemporary COG trials, both EFS and OS were significantly worse for SR patients with iAMP21 compared with those without iAMP21, although this was not the case for HR patients. The treatment approach differed for the SR and HR groups, with SR patients with rapid initial responses (SR-low and SR-average subgroups) receiving less intensive therapy than HR patients (Data Supplement). Therapy for SR patients with a slow early response (SR high) was intensified to include two augmented interim maintenance and delayed intensification phases, mirroring the postinduction therapy administered to those with NCI HR ALL. All HR patients received the COG augmented BFM regimen shown to be superior to the prior standard regimen in Children's Cancer Group (CCG) -1882 and CCG-1961.[19,20] All patients were randomly assigned to receive dexamethasone 10 mg/m^2 per day from days 1 through 14 versus

Table 2. Four-Year EFS Rates by iAMP21 Status and End Induction MRD Status

Variable	Non-iAMP21		iAMP21	
	MRD < 0.01%	MRD ≥ 0.01%	MRD < 0.01%	MRD ≥ 0.01%
Pooled SR and HR patients				
EFS	92.2 ± 0.5	77.3 ± 1.6	84.3 ± 6.4	58.7 ± 9.7
OS	96.6 ± 0.4	88.6 ± 1.2	94.0 ± 4.3	82 ± 7.8
SR patients				
EFS	94.2 ± 0.5	85.2 ± 9.0	82.2 ± 9.1	59.6 ± 12.9
OS	98.1 ± 0.3	93.8 ± 6.2	93.0 ± 6.2	85.2 ± 9.1
HR patients				
EFS	87.9 ± 1.1	67.0 ± 2.6	85.9 ± 9.3	58.0 ± 16.8
OS	93.1 ± 0.9	81.7 ± 2.1	94.7 ± 6.0	78.4 ± 13.8

NOTE. Log-rank $P < .001$.
Abbreviations: EFS, event-free survival; HR, high risk; iAMP21, intrachromosomal amplification of a region of chromosome 21; MRD, minimal residual disease; OS, overall survival; SR, standard risk.

Table 4. Summary of First Events in iAMP21 Patients

Event Type	Standard Risk (n = 74)		High Risk (n = 84)	
	No.	%	No.	%
Induction failure	1	5.6	1	7.1
Induction death	0	0	3	21.4
Relapse	16	88.8	9	64.4
Isolated marrow	7	38.8	6	43.0
Isolated CNS	5	27.7	2	14.3
Testicular	1	5.6	0	0
Marrow + extramedullary	3	16.7	1	7.1
Second malignant neoplasm	1	5.6	0	0
Death in remission	0	0	1	7.1
Total events	18		14	

Abbreviation: iAMP21, intrachromosomal amplification of a region of chromosome 21.

prednisone 60 mg/m^2 per day for days 1 through 28 during induction and high-dose methotrexate with leucovorin rescue versus increasing dose of Capizzi methotrexate without rescue plus polyethylene glycol (PEG) –asparaginase during the first interim maintenance phase. After June 2008, all patients age ≥ 10 years at diagnosis were nonrandomly assigned to prednisone during induction therapy because of a higher-than-expected incidence of osteonecrosis in the older age group with dexamethasone. Similar to the approach taken for SR patients with ALL, therapy was augmented for HR patients with slow early treatment responses. Our finding that iAMP21 did not significantly affect outcomes in HR patients, whereas it did among SR patients with ALL treated less intensively extends the findings of the older BFM and United Kingdom ALL trials.[9-11] In further support of the benefit of augmented therapy for this subgroup, the outcomes of SR high iAMP21 patients with slow early responses (day 15 marrow M2/3 and/or end induction MRD ≥ 0.1%), who were nonrandomly assigned to augmented BFM-based postinduction therapy were similar to those observed in low- and average-risk SR iAMP21 patients with rapid initial responses who received standard-intensity SR therapy (Data Supplement), and to those with HR ALL and iAMP21 who had a slow early response. Taken together, these data establish that iAMP21 patients should be treated with more intensive regimens and not with less intensive therapy as was used in COG AALL0331, regardless of their MRD response. Consequently, current COG ALL trials assign SR iAMP21 patients to the HR ALL study following completion of induction therapy.

Table 3. Multivariate Cox Regression Analyses Among All Patients (pooled high risk and standard risk)

Parameter	Hazard Ratio	95% CI	*P*
iAMP21	1.365	0.937 to 1.988	.1050
Age ≥ 10 years	1.761	1.509 to 2.056	< .001
WBC ≥ 50,000/μL	1.791	1.518 to 2.114	< .001
Day 29 MRD ≥ 0.01%	2.693	2.321 to 3.123	< .001
Known to be *ETV6-RUNX1* and/or have trisomies of chromosomes 4 and 10	0.430	0.357 to 0.519	< .001

Abbreviations: iAMP21, intrachromosomal amplification of a region of chromosome 21; MRD, minimal residual disease.

The test for interaction between iAMP21 and MRD status was not significant in the COG studies. We found that MRD and iAMP21 were independently prognostic in SR ALL, but only MRD was predictive of outcome in HR ALL. These clinical trials did not incorporate HSCT in first remission. Given that iAMP21 was only significant in multivariate analyses for SR patients treated with relatively modest intensity chemotherapy, our data establish that there is no role for routine use of allogeneic HSCT in first remission for patients with iAMP21 on the basis of iAMP21 status alone. Attarbaschi et al[9] reported that MRD was a robust predictor of outcome in iAMP21 patients in the Austrian and German ALL-BFM 86, 90, 95 and 2000 trials. On those trials, the nine iAMP21 patients who were MRD negative by PCR for detection of clone-specific immunoglobulin and T-cell receptor gene rearrangements at days 33 and 78 had a 100% relapse-free survival, whereas those who were MRD positive at either time point had a relapse-free survival of only 37% ± 16% ($P = .02$). Eight of the 11 patients who relapsed had MRD results available; seven were classified as intermediate risk and one as HR by MRD analysis.[9] In our study, SR patients with positive day 29 MRD and iAMP21 had the poorest EFS and OS, and those with neither had the best outcome. In contrast, the presence of iAMP21 did not significantly alter the outcomes of MRD-positive or MRD-negative HR patients.

The biologic basis of iAMP21 remains to be elucidated. It appears to be the primary genetic event in these cases.[12] *RUNX1* is in the common region of amplification, but it is neither mutated nor overexpressed.[13] Other genes identified in the region include microRNA-802 (miR-802) and genes of the Down syndrome critical region.[12] Interestingly, in these patients, the abnormal chromosome 21 frequently also has a region of deletion at the terminus.[13,21] The impact of this latter aberration on outcome is not known. Further identification of the genes in these regions and their functions may help to develop novel therapies for patients with iAMP21.

In conclusion, patients with iAMP21 have inferior outcomes with the less intensive chemotherapy regimens used in contemporary SR COG ALL trials, even if they had MRD less than 0.01% at end induction, and they are not appropriate candidates for the lower intensity therapy that is highly effective in other SR patients with ALL.

In contrast, the outcome of HR iAMP21 patients was not significantly different from those lacking this somatic genetic lesion. On the basis of these findings, the COG now treats all patients with iAMP21, regardless of NCI risk group or MRD response, with an HR postinduction therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** None **Stock Ownership:** None **Honoraria:** None **Research Funding:** Michael J. Borowitz, Becton Dickinson, Beckman Coulter; Julie M. Gastier-Foster, Bristol-Myers Squibb **Expert Testimony:** None **Patents:** None **Other Remuneration:** None

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